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ORIGINAL REPORT

Human Papillomavirus Antibodies and Future Risk of Anogenital Cancer: A Nested Case-Control Study in the European Prospective Investigation Into Cancer and Nutrition Study

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ABSTRACT

Purpose
Human papillomavirus (HPV) type 16 (HPV16) causes cancer at several anatomic sites. In the European Prospective Investigation Into Cancer and Nutrition study, HPV16 E6 seropositivity was present more than 10 years before oropharyngeal cancer diagnosis and was nearly absent in controls. The current study sought to evaluate the extent to which HPV16 E6 antibodies are present before diagnosis of anogenital cancers within the same cohort.

Methods
Four hundred incident anogenital cancers (273 cervical, 24 anal, 67 vulvar, 12 vaginal, and 24 penile cancers) with prediagnostic blood samples (collected on average 3 and 8 years before diagnosis for cervix and noncervix cancers, respectively) and 718 matched controls were included. Plasma was analyzed for antibodies against HPV16 E6 and multiple other HPV proteins and genotypes and evaluated in relation to risk using unconditional logistic regression.

Results
HPV16 E6 seropositivity was present in 29.2% of individuals (seven of 24 individuals) who later developed anal cancer compared with 0.6% of controls (four of 718 controls) who remained cancer free (odds ratio [OR], 75.9; 95% CI, 17.9 to 321). HPV16 E6 seropositivity was less common for cancers of the cervix (3.3%), vagina (8.3%), vulva (1.5%), and penis (8.3%). No associations were seen for non–type 16 HPV E6 antibodies, apart from anti-HPV58 E6 and anal cancer (OR, 6.8; 95% CI, 1.4 to 33.1). HPV16 E6 seropositivity tended to increase in blood samples drawn closer in time to cancer diagnosis.

Conclusion
HPV16 E6 seropositivity is relatively common before diagnosis of anal cancer but rare for other HPV-related anogenital cancers.

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INTRODUCTION

Human papillomavirus (HPV) type 16 (HPV16) causes approximately 50% of cervical cancers, 80% of anal cancers, and roughly half of vaginal, vulvar, and penile cancers worldwide.[1-5] The fraction of oropharyngeal cancers (OPCs) caused by HPV16 varies greatly by geographic region; approximately 60% to 70% of OPCs in some developed countries are caused by HPV16 compared with a much smaller proportion (< 10%) in developing countries.[6-10] Recently, we reported that patients with HPV16 E6 seropositivity were at greater than 200-fold increased risk of OPC, and these antibodies were present up to 10 years before diagnosis, while being extremely rare among cancer-free controls.[11] These results were noteworthy because they suggest that it might be possible to develop a highly specific biomarker for HPV-driven OPC that may be useful for screening,[12] at least if the current OPC incidence trends continue to increase.[6]

Previous case-control studies have reported associations between HPV16 E6 seropositivity and anogenital cancers, specifically among cancers of the uterine cervix[13-17] and penis[18]; these studies were retrospective, with blood samples collected at the time of diagnosis. Three prospective studies on HPV16 E6 seropositivity and anogenital cancer have been conducted to date, two for cervical cancer[19,20] and one for anal cancer.[21] These studies identified associations between HPV16 E6 seropositivity and cancer development, with seropositivity more frequently detected a few years before diagnosis. We aimed to clarify the association between HPV16 E6 antibody positivity and risk of anogenital cancers, including incident cervical, anal, penile, vulvar, and vaginal cancers, within the European Prospective Investigation Into Cancer and Nutrition (EPIC) study.[22]

METHODS

Study Cohort

The EPIC cohort was designed to investigate the relationship between nutritional and lifestyle factors and incidence of cancer and other chronic diseases.[22] Questionnaire data were collected between 1992 and 2000 from 521,330 individuals across Europe, of whom 385,747 provided a blood sample. All participants gave written informed consent, and the research was approved by the local ethics committees and the International Agency for Research on Cancer Institutional Review Board.

Follow-Up for Cancer Incidence

Incident cancers were identified through population-based cancer registries (Denmark, Italy [except Naples], the Netherlands, Norway, Spain, Sweden, and the United Kingdom) or by active follow-up (France, Germany, Greece, and Naples). Active follow-up involved a combination of methods, including review of health insurance records and cancer and pathology registries, as well as direct contact with participants and their next of kin.

Selection of Patient Cases and Controls

We identified 1,829 patients with histologically confirmed anogenital cancer without a history of another cancer (except nonmelanoma skin cancer), defined using the International Classification of Diseases for Oncology, Second Edition (ICD-O-2), including invasive cancer of the cervix uteri (ICD-O-2 C53.0 to C53.9), anus (ICD-O-2 C21.1), vulva (ICD-O-2 C51.0 to C51.9), vagina (ICD-O-2 C52.9), and penis (ICD-O-2 C60.0 to C60.9). After excluding prevalent patients (n = 122), patients without available blood samples (n = 893), patients without baseline questionnaire (n = 1), and patients from three centers that did not participate in the current study (Copenhagen, Århus, and Malmö, n = 253), 560 eligible patients remained. We included all eligible patients with noncervical anogenital cancer (n = 127), including 24 anal cancers, 67 vulvar cancers, 12 vaginal cancers, and 24 penile cancers. Many more eligible patients with cervical cancer were available than for the other cancer sites (n = 443), and because previous studies[18,19] indicated that HPV seroconversion occurred closer to diagnosis for cervical cancer, we selected a subset of 200 patients by oversampling among those with shorter time from blood draw to diagnosis (lead time). The final study population also included all additional eligible cervical cancers from the Swedish Umeå center (n = 73), adding up to a total of 273 cervical cancers, 34 with a lead time between 0 and less than 1 year ($n_{available}$ = 59), 52 with a lead time between 1 and less than 2 years ($n_{available}$ = 61), 99 with a lead time between 2 and less than 5 years ($n_{available}$ = 171), 71 with a lead time between 5 and less than 10 years ($n_{available}$ = 152), and 17 with a lead time of ≥ 10 years ($n_{available}$ = 42).

For each patient case, two controls were randomly chosen from appropriate risk sets consisting of all cohort members alive and free of cancer (except nonmelanoma skin cancer) at the time (and hence age) of diagnosis of the index patient case. Matching criteria included study center, sex, date of blood collection (± 3 months, relaxed to ± 6 months for sets without available controls), age at blood collection (± 3 months, relaxed to ± 2 years for sets without available controls), fasting status, and where relevant, menopausal status, postmenopausal hormone replacement therapy use, and menstrual cycle. The final study population included a total of 273 patients with cervical cancer and 127 patients with noncervical cancer, along with 718 controls.

Serologic Analyses

Plasma samples were sent on dry ice to the German Cancer Research Center (Heidelberg, Germany) and stored at −20°C until analysis. Testing was performed using multiplex assays[23,24] by laboratory staff blinded to the case-control status of the participants. Antigens were affinity-purified, bacterially expressed fusion proteins with N-terminal glutathione *S*-transferase. Samples were analyzed for antibodies to the major capsid protein (L1), the early oncoproteins (E6 and E7), and other early proteins (E1, E2, and E4) of the following carcinogenic mucosal types: HPV16 and HPV18 (L1, E1, E2, E4, E6, and E7) and HPV31, HPV33, HPV45, and HPV52 (L1, E6, and E7). We also analyzed the noncarcinogenic mucosal types HPV6 and HPV11 (L1, E6, and E7). We used the same median fluorescence intensity (MFI) cutoffs as in our previous analysis when defining HPV-seropositive patients.[11] A subset of samples were randomly chosen and included as blinded duplicates; intraclass correlation coefficients ranged from 95% to 98%, and coefficients of variation were less than 5%.

Statistical Analyses

Characteristics of the patients with cancer (by anatomic site of the cancer) and controls were tabulated. Odds ratios (ORs) and 95% CIs were calculated by anatomic site using unconditional logistic regression (because few controls were seropositive for some markers, the final risk analysis included all controls to allow calculation of the OR). Covariates in the models comprised the following matching factors: country (North *v* South Europe, where northern Europe included Denmark, Germany, Great Britain, the Netherlands, and Sweden and southern Europe included France, Greece, Italy, and Spain), sex (for anal cancer, the only anatomic site that occurs in both sexes), and age (as a continuous variable). Additional adjustment by tobacco did not meaningfully affect the point estimates. Further statistical adjustment was not possible because of the limited sample numbers and rarity of HPV16 E6–positive patients and controls. MFI values were evaluated among HPV16 E6–positive individuals. Data on cancer stage were only available for approximately 40% of patients and were not considered in the analysis.

In our previous work,[11] higher specificity was achieved without loss of sensitivity when applying a more stringent threshold for HPV16 E6 seropositivity. Thus, we similarly evaluated the validity in prediction of anogenital cancer after increasing the MFI cutoff of 484 to 1,000 for considering study participants seropositive.

RESULTS

Baseline Characteristics

From the EPIC cohort, incident cervical (n = 273), anal (n = 24), vulvar (n = 67), vaginal (n = 12), and penile (n = 24) cancers and 718 cancer-free individuals were included (Table 1). The patients with cancer were generally representative of those not included with respect to age, sex (anal cancer only), smoking, and education (Appendix Table A1, online only). Of the patients with anal cancer (the only cancer site under study that occurred in both sexes), 87.5% were women. Among patient cases, median age at diagnosis was younger for patients with cancer of the cervix (43.0 years) compared with patients with cancers of the anus (61.0 years), vagina/vulva (65.0 years), and penis (63.5 years). The median time between blood draw and diagnosis (lead time) ranged from 7 to 8 years for the noncervix cancer sites. Because patients with cervical cancer with shorter lead time were oversampled, the median lead time for cervical cancer was 3 years.

Table 1. Demographics and Clinical Characteristics of the Analyzed Population

Demographic or Clinical Characteristic	Controls				Patients With Cancer									
	Male (n = 60)		Female (n = 658)		Cervical (n = 273)		Anal (n = 24)		Penile (n = 24)		Vaginal (n = 12)		Vulvar (n = 67)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Sex														
Male	60	100	0	0	0	0	3	12.5	24	100	0	0	0	0
Female	0	0	658	100	273	100	21	87.5	0	0	12	100	67	100
Age, years														
≤ 40	2	3.3	248	37.7	166	60.8	3	12.5	1	4.2	1	8.3	6	9.0
41-50	17	28.3	167	25.4	66	24.2	7	29.2	7	29.2	3	25.0	10	14.9
51-60	21	35.0	133	20.2	28	10.3	5	20.8	9	37.5	5	41.7	26	38.8
61-70	16	26.7	97	14.7	11	4.0	8	33.3	5	20.8	3	25.0	23	34.3
> 70	4	6.7	13	2.0	2	0.7	1	4.2	2	8.3	0	0	2	3.0
Country														
France	0	0	10	1.5	0	0	2	8.3	0	0	0	0	1	1.5
Germany	20	33.3	154	23.4	45	16.5	9	37.5	7	29.2	6	50.0	12	17.9
Great Britain	16	26.7	138	21.0	49	17.9	5	20.8	5	20.8	0	0	13	19.4
Greece	4	6.7	40	6.1	10	3.7	0	0	2	8.3	1	8.3	7	10.5
Italy	6	10.0	62	9.4	18	6.6	3	12.5	2	8.3	1	8.3	7	10.5
The Netherlands	2	3.3	52	7.9	5	1.8	1	4.2	1	4.2	1	8.3	17	25.4
Norway	0	0	12	1.8	2	0.7	0	0	0	0	1	8.3	2	3.0
Spain	8	13.3	57	8.7	22	8.1	0	0	4	16.7	1	8.3	5	7.5
Sweden	4	6.7	134	20.2	122	44.7	4	16.7	3	12.5	1	8.3	3	4.5
Smoking														
Never	18	30.0	391	59.4	127	46.5	12	50.0	8	33.3	10	83.3	36	53.7
Former	29	48.3	136	20.7	59	21.6	6	25.0	8	33.3	1	8.3	14	20.9
Current	13	21.7	121	18.4	83	30.4	4	16.7	8	33.3	1	8.3	17	25.4
Education														
Primary	17	28.3	216	32.9	54	19.8	11	45.8	10	41.7	6	50.0	32	47.8
> Primary school	43	71.7	434	66.0	216	79.1	11	45.8	14	58.3	6	50.0	35	52.2

NOTE. All variables were assessed at enrollment. Percentages do not add to 100% as a result of missing data.

HPV16 Seropositivity and Cancer Risk

Seropositivity against HPV16 E6 was most frequent in prediagnostic plasma from patients with anal cancer (seven of 24 patients; 29.2%; Table 2). HPV16 E6 seroprevalence was substantially lower in all other anogenital cancers, including cancer of the cervix (nine of 274 patients; 3.3%), penis (two of 24 patients; 8.3%), vagina (one of 12 patients; 8.3%), and vulva (one of 67 patients; 1.5%). The anatomic subsites of the HPV16 E6–seropositive penile cancers were prepuce (ICD-O-2 C60.0) and glans penis (ICD-O-2 C60.1), and the vulvar cancer was vulva, not otherwise specified (ICD-O-2 C51.9). HPV16 E6 seropositivity was extremely rare in cancer-free individuals (four of 718 individuals; 0.6%; Table 2).

Of the six HPV16 proteins evaluated, E6 was most strongly associated with all anogenital cancer sites; anal cancer displayed the strongest association, with an OR of 75.9 (95% CI, 17.9 to 321), followed by vaginal cancer, with an OR of 24.1 (95% CI, 2.1 to 277), and cervical cancer, with an OR of 9.5 (95% CI, 2.4 to 37.1). Similarly, HPV16 L1 seropositivity was also associated with risk of anal (OR, 11.0; 95% CI, 4.7 to 25.7), vulvar (OR, 3.4; 95% CI, 1.8 to 6.4), and cervical (OR, 2.9; 95% CI, 1.9 to 4.2) cancers. Risk of anal cancer was elevated for most of the HPV16 proteins, in contrast to the other anatomic sites; beyond E6 and L1, an increased risk of anal cancer was observed for HPV16 E7 (OR, 7.3; 95% CI, 2.9 to 18.4) and E1 (OR, 4.5; 95% CI, 1.6 to 12.8) seropositivity, but not for E2 or E4 (Table 2). All seven of the HPV16 E6 seropositive anal cancers occurred among women (n = 21; HPV16 E6 seroprevalence among women with anal cancer was 33.3%); none of the three men with anal cancer had HPV16 E6 seropositivity. No differences were seen between HPV16 E6–positive and –negative anal cancers by other variables (including age at diagnosis, region of Europe [south *v* north], smoking or drinking status, or body mass index; data not shown).

In the sensitivity analysis using the more stringent definition of HPV16 E6 seropositivity (MFI, 1,000), all of the HPV16 E6–seropositive anal, vaginal, and vulvar cancers (using the lower threshold) remained positive, whereas seven of the nine cervical cancers, one of the two penile cancers, and one of the four controls remained positive at the higher threshold.

MFI values were evaluated among HPV16 E6–positive individuals. HPV16 E6–seropositive controls seemed to have lower median values (MFI, 788) than did HPV16 E6–seropositive patients with anal (MFI, 2,694), cervical (MFI, 3,400), vaginal (MFI, 5,761), and vulvar (MFI, 1,914) cancer, but not penile cancer (MFI, 970).

HPV16 E6 Seropositivity and Time to Diagnosis of Cancer

HPV16 E6 seropositivity and cancer risk were evaluated in two strata defined by lead time between blood collection and cancer diagnosis (< or ≥ 5 years; Table 3). Of the eight anal cancers diagnosed in the interval with less than 5 years between blood draw and diagnosis, 62.5% (n = 5) were HPV16 E6 seropositive, whereas of the 16 anal

Table 2. Risk of Anogenital Cancer Associated With HPV16 Serology Status After Adjustment for Matching Factors

	Controls (n = 719)		Cervix (n = 273)				Anus (n = 24)				Penis (n = 24)				Vagina (n = 12)				Vulva (n = 67)			
			Patients				Patients				Patients				Patients				Patients			
Serology	No.	%	No.	%	OR	95% CI	No.	%	OR	95% CI	No.	%	OR	95% CI	No.	%	OR	95% CI	No.	%	OR	95% CI
E6																						
Negative	715	99.4	264	96.7	1.0		17	70.8	1.0		22	91.7	1.0		11	91.7	1.0		66	98.5	1.0	
Positive	4	0.6	9	3.3	9.5	2.4 to 37.1	7	29.2	75.9	17.9 to 321	2	8.3	5.4	0.5 to 63.4	1	8.3	24.1	2.1 to 277	1	1.5	4.0	0.4 to 46.0
E7																						
Negative	678	94.3	251	92	1.0		16	66.7	1.0		21	87.5	1.0		12	100	NE		62	92.5	1.0	
Positive	41	5.7	22	8	1.6	0.9 to 2.7	8	33.3	7.3	2.9 to 18.4	3	12.5	2.8	0.5 to 15.9	0	0			5	7.5	1.1	0.4 to 2.9
L1																						
Negative	648	90.1	209	76.6	1.0		11	45.8	1.0		20	83.3	1.0		9	75.0	1.0		49	73.1	1.0	
Positive	71	9.9	64	23.4	2.9	1.9 to 4.2	13	54.2	11.0	4.7 to 25.7	4	16.7	1.8	0.4 to 7.0	3	25.0	3.0	0.8 to 11.4	18	26.9	3.4	1.8 to 6.4
E1																						
Negative	680	94.6	252	92.3	1.0		19	79.2	1.0		22	91.7	1.0		12	100	NE		64	95.5	1.0	
Positive	39	5.4	21	7.7	1.5	0.8 to 2.7	5	20.8	4.5	1.6 to 12.8	2	8.3	1.0	0.2 to 5.8	0	0			3	4.5	0.9	0.2 to 3.0
E2																						
Negative	685	95.3	261	95.6	1.0		21	87.5	1.0		21	87.5	1.0		12	100	NE		62	92.5	1.0	
Positive	34	4.7	12	4.4	1.0	0.5 to 2.0	3	12.5	2.8	0.8 to 10.1	3	12.5	3.2	0.6 to 18.1	0	0			5	7.5	1.5	0.5 to 4.3
E4																						
Negative	595	82.8	223	81.8	1.0		17	70.8	1.0		20	83.3	1.0		12	100	NE		56	83.6	1.0	
Positive	124	17.2	50	18.2	1.0	0.7 to 1.5	7	29.2	2.1	0.8 to 5.3	4	16.7	1.1	0.3 to 4.1	0	0			11	16.4	1.0	0.5 to 1.9

Abbreviations: HPV16, human papillomavirus type 16; NE, not estimable; OR, odds ratio.

Table 3. HPV16 E6 Serology Status by Time Between Blood Collection and Diagnosis Among HPV16 E6–Seropositive Patients Only

Time Interval (years)	Cervix			Anus			Penis			Vagina			Vulva		
	No. of Patients in Interval	HPV16 E6 Positive		No. of Patients in Interval	HPV16 E6 Positive		No. of Patients in Interval	HPV16 E6 Positive		No. of Patients in Interval	HPV16 E6 Positive		No. of Patients in Interval	HPV16 E6 Positive	
		No.	%		No.	%		No.	%		No.	%		No.	%
< 5	185	8	4.3	8	5	62.5	7	1	14.2	4	0	0	21	1	4.7
≥ 5	88	1	1.1	16	2	12.5	17	1	5.8	8	1	12.5	46	0	0

Abbreviation: HPV16, human papillomavirus type 16.

cancers diagnosed with a longer lead time, only 12.5% (n = 2) were HPV16 E6 seropositive. For patients with cervical cancer, HPV16 E6 seropositivity was more common with the shorter lead time (eight of 185 patients; 4.3%) versus the longer lead time (one of 88 patients; 1.1%). When the shorter interval was further stratified for cervical cancer (the only site with sufficient sample size), HPV16 E6 seropositivity increased the closer in time to cancer diagnosis, being 8.8% (three of 34 patients), 3.8% (two of 52 patients), and 3.0% (three of 99 patients) for 0 to less than 1, 1 to less than 2, and 2 to less than 5 years before diagnosis, respectively.

Non–Type 16 HPV E6 Seropositivity and Cancer Risk

Among the non-HPV16 E6 proteins, associations between HPV33 and HPV58 and anal cancer were observed; the odds increased 17-fold (95% CI, 3.9 to 79.2) for HPV33 and seven-fold (95% CI, 1.9 to 27.7) for HPV58 (Table 4). Because these HPV types are phylogenetically related to HPV16, these associations were further analyzed restricted to patients with anal cancer and controls who were HPV16 E6 seronegative to eliminate any effects caused by antibody cross-reactivity[13]; the significant association persisted for HPV58 (OR, 6.8; 95% CI, 1.4 to 33.1) but not for HPV33 (OR, 7.4; 95% CI, 0.8 to 67.2). No clear associations were observed for cervical cancer and non-16 HPV types (Table 4).

DISCUSSION

In this comprehensive analysis of HPV-related anogenital cancers, we observed that HPV16 E6 seropositivity was common in patients who later developed anal cancer. For anal cancer, 29% of patients and 0.6% of controls were seropositive for HPV16 E6, corresponding to a 75-fold risk increase in HPV16 E6–seropositive versus–seronegative individuals. We also observed increased risk among HPV16 E6–seropositive individuals for cancers of the vagina, cervix, and penis, but the magnitude of the associations was not nearly as strong as for anal cancer.

Few studies have prospectively investigated HPV16 E6 and risk of anogenital cancer. One study of cervical cancer (median follow-up time, 3.5 years) showed that 4% of patients had prediagnostic antibodies to the HPV16/18 E6 or E7 proteins compared with 1.2% of controls. Increased risk was only evident for cervical cancer diagnosed within approximately 3 years of blood draw.[19] In a more recent study, Castellsagué et al[20] observed that 11% of incident invasive cervical cancers from EPIC (a subset [n = 60, 22%] of the patients in the current analysis) were HPV16 E6 seropositive compared with 1.4% of controls. The proportions seropositive were higher than what were observed in the current analysis (patients, 3.3%; controls, 0.6%), but the magnitude of the association was similar, suggesting that a more stringent threshold for positivity was used in the current analysis. Furthermore, HPV16 E6 seroprevalence further increased in those with shorter time between blood draw and diagnosis, with 9% of cervical cancers diagnosed within a year of blood draw being HPV16 E6 seropositive. For anal cancer, Bertisch et al[21] showed that 22% of incident anal cancers among HIV-positive individuals had prediagnostic HPV16 E6 seropositivity, a result that would seem consistent with our overall estimate of 29%; yet in our samples taken closer to diagnosis (< 5 years; Table 3), a notably higher fraction were positive.

The observed strong association between HPV16 E6 serology and anal cancer should be interpreted in the context of our previous study on head and neck cancer within the same cohort,[11] as well as the published literature. During the period of time of the EPIC study (1990s to 2000s), a notably higher fraction of anal cancers (approximately 63%)[5] than OPCs (approximately 35%)[25] were likely caused by HPV16 infection. Thirty-five percent of OPCs in our previous study were HPV16 E6 seropositive[11]; these data suggest that the vast majority of HPV-driven OPCs were identified in the prediagnostic sera. In contrast, only 29% of incident anal cancers were HPV16 E6 seropositive in EPIC, which is a notably lower fraction than observed in previous studies of HPV16 DNA tumor prevalence in anal cancer.[5] We interpret this to mean that the vast majority of HPV-driven OPCs seroconvert well before clinical manifestation, whereas some, but not all, anal cancers do, and that HPV serology would be more sensitive for detection of HPV-driven OPC than for HPV-driven anal cancer. In support of this, we also noted that most of the HPV16 E6–seropositive anal cancers were diagnosed less than 5 years after blood draw, whereas the fraction of seropositive OPCs was stable over the ≥ 10-year follow-up period.[11]

Our results further indicate that a preclinical antibody response is mostly lacking for patients with cervical, penile, vaginal, and vulvar cancer when assessed within the same cohort using the same laboratory and assay for serologic testing. A recent cross-sectional study demonstrated that, for cervical cancer, HPV16 E6 seropositivity at time of diagnosis was 32% among all invasive cervical cancers (and 50% among HPV16 DNA–positive invasive cervical cancers)[12]; HPV E6 and E7 antibodies may be late tumor markers that increase with clinical tumor stage.[14,15,17] Our limited data on cervical cancers diagnosed shortly after blood draw also suggested a higher HPV16 seroprevalence immediately preceding diagnosis, which decreases as one moves further from diagnosis (8.8% in the year preceding diagnosis; 3.8% in blood collected 1 to 2 years before diagnosis), again highlighting differences in the immune response by anatomic site.

Table 4. Risk of Anogenital Cancer Associated With Non–HPV16 E6 Serology Status

	Controls		Cervix				Anus				Penis				Vagina				Vulva			
			Patients				Patients				Patients				Patients				Patients			
Serology	No.	%	No.	%	OR	95% CI	No.	%	OR	95% CI	No.	%	OR	95% CI	No.	%	OR	95% CI	No.	%	OR	95% CI
HPV6																						
Negative	718	99.9	273	100	NE		24	100	NE		24	100	NE		12	100	NE		67	100	NE	
Positive	1	0.1	0	0			0	0			0	0			0	0			0	0		
HPV11																						
Negative	714	99.3	267	97.8	1.0		24	100	NE		24	100	NE		12	100	NE		67	100	NE	
Positive	5	0.7	6	2.2	3.1	0.9 to 10.4	0	0			0	0			0	0			0	0		
HPV18																						
Negative	711	98.9	267	97.8	1.0		24	100	NE		23	98.9	1.0		12	100	NE		66	98.5	1.0	
Positive	8	1.1	6	2.2	2.1	0.7 to 6.5	0	0			1	4.2	2.5	0.2 to 42.5	0	0			1	1.5	1.4	0.2 to 12.1
HPV31																						
Negative	711	98.9	269	98.5	1.0		23	95.8	1.0		24	100	NE		11	91.7	1.0		66	98.5	1.0	
Positive	8	1.1	4	1.5	1.1	0.3 to 3.9	1	4.2	4.3	0.5 to 37.8	0	0			1	8.3	8.3	0.9 to 78.1	1	1.5	1.3	0.2 to 11.3
HPV33																						
Negative	713	99.2	266	98.2	1.0		21	87.5	1.0		24	100	NE		12	100	NE		66	98.5	1.0	
Positive	6	0.8	7	2.6	2.8	0.9 to 8.4	3	12.5	17.5	3.9 to 79.2	0	0			0	0			1	1.5	2.4	0.3 to 20.5
HPV45																						
Negative	701	97.5	268	98.2	1.0		24	100	NE		24	100	NE		12	100	NE		66	98.5	1.0	
Positive	18	2.5	5	1.8	1.0	0.3 to 2.9	0	0			0	0			0	0			1	1.5	0.5	0.1 to 4.2
HPV58																						
Negative	702	97.6	268	98.2	1.0		21	87.5	1.0		23	98.9	NE		12	100	NE		67	100	NE	
Positive	17	2.4	5	1.8	0.7	0.3 to 2.1	3	12.5	7.2	1.9 to 27.7	1	4.2			0	0			0	0		

Abbreviations: HPV, human papillomavirus; NE, not estimable; OR, odds ratio.

It is presently unclear why the associations between HPV16 E6 and cancer differ by anatomic site, but the immunobiology and proximity to the lymphatic system (and thus access to antigen-presenting cells) are likely important. The tonsil is a lymphoid organ rich in immune cells including antibody-producing B cells. Similarly, in the anus, HPV infection occurs at the dentate (pectinate) line or transformation zone of squamous and nonsquamous mucosa. This site is in close proximity to the secondary lymphoid tissue of the GI tract including Peyer's, cecal, and rectal patches, as well as isolated lymphoid follicles, which are rich in immune cells and can also serve as sites of induction of immune responses including antibody responses. However, humoral immunity is more difficult to induce in the female genital tract,[26,27] given that the reproductive tract must maintain the delicate balance between inhibiting immune responses to spermatozoa and fetuses and inducing immunity against foreign microbes. In fact, most of the immunity present within the reproductive tract is derived from other mucosal sites, predominately the rectum and Peyer's patches of the GI tract.[14,15] If cervical immunity is mostly local and does not engage systemic humoral immunity, antibody responses to biologically relevant infections will be muted, consistent with our data. Furthermore, the penis and vulva are largely keratinized epithelia, which may further reduce access to the immune system compared with infections that occur at mucosal surfaces.

The main limitation of this work was that the number of incident cancers available for study was relatively small, an inherent issue in studies of rare cancers. Furthermore, subsequent work will involve identifying tumor material from prospectively detected cases to evaluate the sensitivity of the serology assay in detecting truly HPV16-driven disease, an undertaking well beyond the scope of the current project. Finally, data on cancer stage were missing in the majority of patients, and thus, we were unable to evaluate whether antibody positivity was related to stage at diagnosis.

Our body of work to date within the European population indicates that seropositivity against HPV16 E6 is common in individuals who later develop OPC, relatively common among those who develop anal cancers, but rare among those who develop other HPV-related cancers, including cervical, vulvar, vaginal, and penile cancers. Nevertheless, seropositivity in cervical cancer, by far the most common of all HPV-related cancers,[25] remained significantly elevated versus controls and increased notably the closer in time to diagnosis. Hence, should the incidence of HPV-related OPC continue increasing to the extent that it would motivate screening using HPV16 E6 serology assay,[8,28] our results suggest that screening for anal and cervical cancers should also be considered in the clinical work-up of patients who test positive for HPV16 E6, in particular in populations lacking adequate screening for cervical cancer. Future studies in populations with different HPV prevalence and studies that aim to understand the preclinical presentation and biologic underpinning of these findings are warranted and will inform on the potential of HPV16 E6 as a prediagnostic biomarker for HPV-driven cancers.

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Disclosures provided by the authors are available with this article at www.jco.org.

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Human Papillomavirus Antibodies and Future Risk of Anogenital Cancer: A Nested Case-Control Study in the European Prospective Investigation Into Cancer and Nutrition Study

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Appendix

Table A1. Comparison of Demographics and Clinical Characteristics of Patients Included and Excluded From the Current Investigation

Demographic or Clinical Characteristic	Cervical Cancer				Anal Cancer				Penile Cancer				Vaginal Cancer				Vulvar Cancer			
	Included (n = 273)		Excluded (n = 793)		Included (n = 24)		Excluded (n = 32)		Included (n = 24)		Excluded (n = 18)		Included (n = 12)		Excluded (n = 6)		Included (n = 67)		Excluded (n = 50)	
	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%
Sex																				
Male	0	0	0	0	3	12.5	5	15.6	24	100	18	100	0	0	0	0	0	0	0	0
Female	273	100	793	100	21	87.5	27	84.4	0	0	0	0	12	100	6	100	67	100	50	100
Age, years																				
≤ 40	96	35.2	232	29.3	0	0	0	0	0	0	0	0	1	8.3	0	0	2	3.0	0	0
41-50	103	37.7	198	25.0	4	16.7	1	3.1	5	20.8	0	0	0	0	0	0	8	11.9	7	14.0
51-60	55	20.1	222	28.0	7	29.2	16	50.0	4	16.7	7	38.9	4	33.3	3	50.0	9	13.4	13	26.0
61-70	13	4.8	114	14.4	10	41.7	10	31.3	8	33.3	8	44.4	6	50.0	2	33.3	33	49.3	16	32.0
> 70	6	2.2	27	3.4	3	12.5	5	15.6	7	29.2	3	16.7	1	8.3	1	16.7	15	22.4	14	28.0
Country																				
Denmark	0	0	94	11.9	0	0	11	34.4	0	0	10	55.6	0	0	1	16.7	0	0	11	22.0
France	0	0	56	7.1	2	8.3	8	25.0	0	0	0	0	0	0	0	0	1	1.5	10	20.0
Germany	45	16.5	51	6.4	9	37.5	1	3.1	7	29.2	0	0	6	50.0	0	0	12	17.9	0	0
Great Britain	49	17.9	346	43.6	5	20.8	5	15.6	5	20.8	1	5.6	0	0	2	33.3	13	19.4	10	20.0
Greece	10	3.7	15	1.9	0	0	0	0	2	8.3	0	0	1	8.3	0	0	7	10.4	0	0
Italy	18	6.6	34	4.3	3	12.5	0	0	2	8.3	0	0	1	8.3	0	0	7	10.4	1	2.0
The Netherlands	5	1.8	20	2.5	1	4.2	0	0	1	4.2	0	0	1	8.3	0	0	17	25.4	0	0
Norway	2	7.0	40	5.0	0	0	5	15.6	0	0	0	0	1	8.3	1	16.7	2	3.0	5	10.0
Spain	22	8.1	31	3.9	0	0	0	0	4	16.7	0	0	1	8.3	1	16.7	5	7.5	0	0
Sweden	122	44.7	106	13.4	4	16.7	2	6.3	3	12.5	7	38.9	1	8.3	1	16.7	3	4.5	13	26.0
Smoking*																				
Never	127	46.5	352	44.4	12	50.0	11	34.4	8	33.3	4	22.2	10	83.3	1	16.7	36	53.7	22	44.0
Former	59	21.6	195	24.6	6	25.0	11	34.4	8	33.3	7	38.9	1	8.3	1	16.7	14	20.9	12	24.0
Current	83	30.4	242	30.5	4	16.7	9	28.1	8	33.3	7	38.9	1	8.3	4	66.7	17	25.4	16	32.0
Education*																				
Primary	54	19.8	143	18.0	11	45.8	3	9.4	10	41.7	7	38.9	6	50.0	4	66.7	32	47.8	12	24.0
> Primary school	216	79.1	639	80.6	11	45.8	29	90.6	14	58.3	11	61.1	6	50.0	2	33.3	35	52.2	36	72.0

NOTE. The following patients were excluded: patients without available blood samples, patients without baseline questionnaire, and patients from three centers that did not participate in the current study (Copenhagen, Århus, and Malmö). All remaining patients with noncervical anogenital cancers were included, as were a subset of the larger number of eligible patients with cervical cancer.

*Percentages do not add to 100% as a result of missing data.